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GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN *VITIS VINIFERA* AND *HYLOCEREUS UNDATUS*

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ABSTRACT

A diet rich in natural antioxidants appears to be inversely associated with degenerative chronic diseases, such as cardiovascular disease, cancer, obesity, and diabetes, as well as with the prevention of general inflammatory health implications. Grapes (Vitis vinifera) and Dragon fruits (Hylocereus undatus) are important antioxidant rich fruits, belonging to the family Vitaceae and Cactaceae, respectively. In the present study, the aqueous extract of Vitis vinifera and Hylocereus undatus were subjected to GC-MS analysis. The major chemical constituents in both fruits were identified.

KEYWORDS: Hylocereus Undatus, Vitis Vinifera & GC-MS Analysis

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Original Article

INTRODUCTION

Grape (*Vitis vinifera*) is one of the most important commercial fruit crops of temperate to tropical regions (Gowda *et al.*, 2008). Grape belongs to the *Vitaceae* family (Jansen *et al.*, 2006), native to the Mediterranean region, central Europe and Southwest Asia and cultivated today in all temperate regions of the world (Gruenwald *et al.*, 2004). Many berries make up a cluster or bunch of grapes. The fruit of the grape is one of the most edible foods, having many established nutritional and medicinal properties for consumers. The grape is gaining popularity for its high nutritive value, excellent in taste, multipurpose use and better returns (Ghosh *et al.*, 2008).

Grape polyphenols range from simple compounds (monomers) to complex tannin type substances (oligomers and polymers). There are many classes of negatively charged polyphenols, identified in grapes, such as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives (Resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myricetin), anthocyanins, etc. (Oliveira *et al.*, 2013). These polyphenols possesses many beneficial effects, on human health such as inhibition of free radical damage, antibacterial, antifungal, decreasing the risk of cardiovascular diseases, anticarcinogenic and anti-inflammatory etc. (Oliveira *et al*., 2013; Daglia 2012; Georgiev *et al.*, 2014 and Kabir *et al.*, 2015).

Dragon fruit or pitaya is one of the tropical fruits, under the cactus family, *Cactaceae*. There are three main types of dragon fruit species, available for commercial cultivation, namely, *Hylocereus undatus* (white flesh with pink skin), *Hylocereus polyrhizus* (red flesh with pink skin) and *Selenicereus megalanthus* (white flesh with yellow skin). *H. undatus* or commonly known as white pitaya, owing to its white flesh (Bellec, 2006; Lim *et al.* 2012). *H. undatus* is generally larger than *H. polyrhizus*, and it weighs 300 to 800 g, with 15 to 22 cm long. *H. undatus* has originated from the southern part of Mexico and it is now widely introduced in Asia countries, such as Taiwan, Malaysia and Vietnam as well as northern Australia (Lim *et al.* 2012). The contents of *H. undatus* are similar to the *H. polyrhizus*. The pitaya flesh contains small black seeds scattered in white-flesh (Barbeau, 1990)

and the raw flesh is mildly sweet and low in calories (Zainoldin and Baba, 2009).

The pitaya fruit has a high nutritional value, and is rich in calcium, phosphorus, potassium and vitamins. Furthermore, it can also be considered as a source of carbohydrates and fibers (Le Bellec *et al.,* 2007; Zainoldin and Baba, 2009). Studies conducted with the pitaya, emphasized its functional properties helping to reduce the risk of chronic diseases and its potential to contribute to physical and mental wellness (Wichienchot *et al.,* 2010). The pulp of the fruit contains antioxidants and oligosaccharides, with prebiotic properties (Wichienchot *et al.,* 2010). Pitaya peel has high quantities of antioxidants (Li-Chen *et al.,* 2006). Moreover, its seeds are rich in essential fatty acids and phytosterols (Ariffin *et al.,* 2009).

Hence, the objective of the present study is to identify the phytochemical constituents of aqueous extract of *Vitis vinifera* and *Hylocereus undatus* fruits, with the aid of GC-MS technique.

MATERIALS AND METHODS

Collection of Fruits

The fresh fruits of *Hylocereus undatus* and *Vitis vinifera* were collected from Koyambedu market, Chennai.

Preparation of Fruit Extract

The fruits were thoroughly washed first with tap water and then distilled water separately. The whole fruits were pureed well, using a juicer and then filtered through a sterilized mesh cloth, to separate the aqueous fraction of fruit of particles. The extracts were concentrated under reduced pressure in a rotary evaporator. The aqueous extracts were used for GC-MS analysis.

Gas Chromatography – Mass Spectrometry (GC – MS) Analysis

An Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used, for sample introduction and the split ratio was set of 10:1. The oven temperature program was programmed to start at 35 C, hold for 2 minutes, then ramp at 20 C per minute, to 300 C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer, operating in electron ionization (EI) mode with TSS-20001 software was used, for all analyses. Low-resolution mass spectra were acquired, at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan, with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750, at 1 second per scan.

Interpretation of mass spectrum GC-MS was conducted, using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The spectrum of the unknown component was compared to the NIST library. The Name, Molecular weight and structure of the components of the test Material, were ascertained. The entire analysis was done in SAIF facility, available at IIT Madras.

RESULTS AND DISCUSSIONS

GC-MS chromatogram of the aqueous extract of *Vitis vinifera* (Figure 1), showed many peaks indicating the

presence of eight major phytochemical constituents. In comparison, of the mass spectra of the constituents with the NIST and WILEY libraries, the phytoconstituents were characterized and identified (Table 1). The major phytochemical constituent mass spectra are Triamcinolone acetonide, 6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine, Oxacycloheptadec-8-en-2-one, 5a-Pregn-16-en-20-one, 3a,12a-dihydroxy-, diacetate, Pseudosolasodinediacetate, Cyclohexanecarboxamide, N-hydroxy-2 (E) -2,4-pentadienyl and Dasycarpidan-1-methanol, acetate (ester).

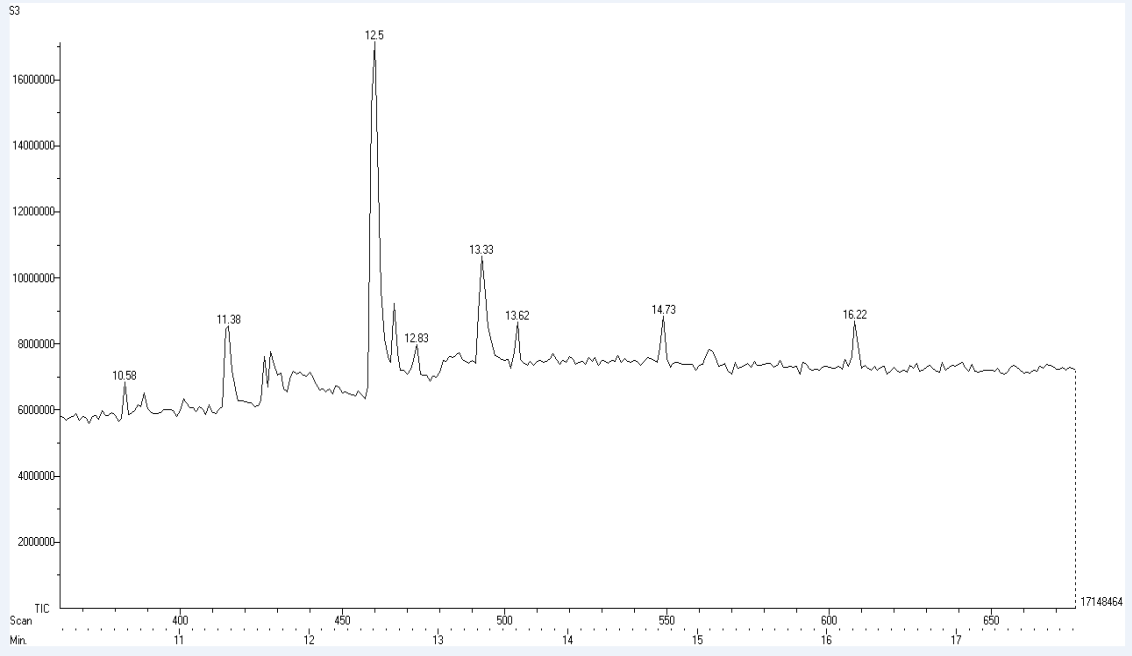

Figure 1: GC-MS Chromatogram of Grape Fruit Extract

Table 1: Phytochemicals Identified in Grape Fruit Extract by GC-MS

RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area %
10.58	Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434.497	8.838392
11.38	6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine	$C_{22}H_{18}BrN$	376.289	11.05707
12.5	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252.392	22.16379
12.83	5a-Pregn-16-en-20-one, 3a,12a-dihydroxy-, diacetate	C23H34O4	416.5503	10.32156
13.33	Pseudosolasodinediacetate	$C_{31}H_{49}NO_4$	499.725	13.75656
13.62	Cyclohexanecarboxamide, N-hydroxy-2 (E) -2,4-pentadienyl	$C_{12}H_{19}NO_2$	209.28476	11.20042
14.73	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.433	11.43133
16.22	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.433	11.23088

Hylocereus undatus showed many peaks in (Figure 2) GC-MS chromatogram, indicating the presence of eight phytochemical constituents. On comparison with mass spectra phyto constituents were characterized and identified (Table 2). The major phytochemical constituent's mass spectra were 6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine, N-[(4,6-Dimethoxynaphthalen-1-yl) methylene] -2,5-dichloro-4-hydroxyphenylamine, Oxacycloheptadec-8-en-2-one, Androstan-17one, 3-ethyl-3-hydroxy-, [5a], Dasycarpidan-1-methanol, acetate (ester), Pyridine-3-carboxamide, 6-chloro-4-trifluoromethyl-N-[2,4-dichloeo-6-methyl] -N-methyl, 1,2-Dipalmitoyl 3-acetyl glycerolGallic acid and Triamcinolone acetonide.

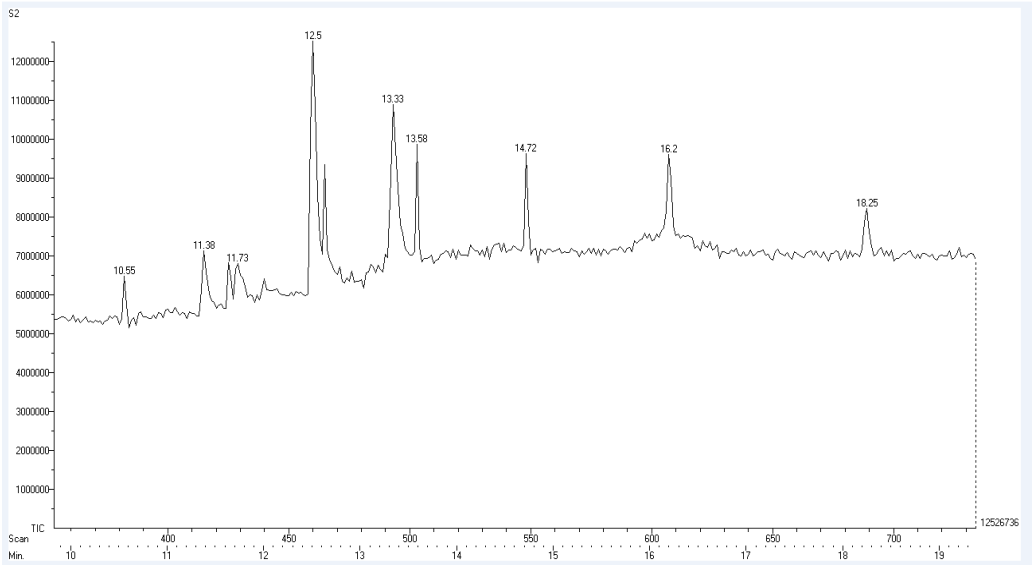

Figure 2: GC-MS Chromatogram of Dragon Fruit Extract

Table 2: Phytochemicals Identified in Dragon Fruit Extract by GC-MS

RT	Compound Name	Molecular Formula	Molecular Weight	Peak Area %
10.55	6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine	$C_{22}H_{18}BrN$	376.289	7.991989
11.38	N-[(4,6-Dimethoxynaphthalen-1-yl)methylene]-2,5-dichloro-4-hydroxyphenylamine	$C_{19}H_{15}Cl_2NO_3$	376.233	8.799596
11.73	6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine	$C_{22}H_{18}BrN$	376.28902	8.383780
12.5	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252.392	15.430418
13.33	Androstan-17one, 3-ethyl-3-hydroxy-, [5a]	$C_{21}H_{34}O_2$	318.49346	13.414466
13.58	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.433	12.154464
14.72	Pyridine-3-carboxamide, 6-chloro-4-trifluoromethyl-N-[2,4-dichloeo-6-methyl] -N-methyl	$C_{15}H_{10}C_{l3}F_3N_2O$	397.606	11.854260
16.2	1,2-Dipalmitoyl 3-acetyl glycerol Gallic acid	$C_{37}H_{70}O_6$	610.948	11.854137
18.25	Triamcinolone acetonide	$C_{24}H_{31}FO_6$	434.497	10.116891

CONCLUSIONS

In the present study, many constituents have been identified from the aqueous fruit extract of *Vitis vinifera* and *Hylocereus undatus*by, GC-MS analysis. Triamcinolone acetonide, Dasycarpidan-1-methanol, acetate (ester), 6-Methyl-2, (4-bromophenyl) -7-phenylmethylindolizine and Oxacycloheptadec-8-en-2-one is present in both fruits. However, further studies are undertaken to study the combined effect of both extracts on animal model, to evaluate their bioactivity.

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